Loose Plant Architecture1, an INDETERMINATE DOMAIN Protein Involved in Shoot Gravitropism, Regulates Plant Architecture in Rice1[W]

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Tiller angle and leaf angle are two important components of rice (Oryza sativa) plant architecture that play a crucial role in determining grain yield. Here, we report the cloning and characterization of the Loose Plant Architecture1 (LPA1) gene in rice, the functional ortholog of the AtHID15/SHOOT GRAVITROPISM5 (SGR5) gene in Arabidopsis (Arabidopsis thaliana). LPA1 regulates tiller angle and leaf angle by controlling the adaxial growth of tiller node and lamina joint. LPA1 was also found to affect shoot gravitropism. Expression pattern analysis suggested that LPA1 influences plant architecture by affecting the gravitropism of leaf sheath pulvinus and lamina joint. However, LPA1 only influences gravity perception or signal transduction in coleoptile gravitropism by regulating the sedimentation rate of amyloplasts, distinct from the actions of LAZY1. LPA1 encodes a plant-specific INDETERMINATE DOMAIN protein and defines a novel subfamily of 28 INDETERMINATE DOMAIN proteins with several unique conserved features. LPA1 is localized in the nucleus and functions as an active transcriptional repressor, an activity mainly conferred by a conserved ethylene response factor-associated amphiphilic repression-like motif. Further analysis suggests that LPA1 participates in a complicated transcriptional and protein interaction network and has evolved novel functions distinct from SGR5. This study not only facilitates the understanding of gravitropism mechanisms but also generates a useful genetic material for rice breeding.

Rice (Oryza sativa) is the staple food for more than half of the world’s population. In the past 50 years, the green revolution and the use of heterosis have greatly improved rice yields (Peng et al., 2008). However, because of the increasing demand for rice production, food security can still be a serious problem. Thus, new elite varieties that can produce much higher grain yields need to be developed, and ideal plant architecture (ideotype) breeding has been shown to be the most promising strategy in tropical areas (Khush, 2005). Rice plant architecture is mainly determined by plant height and tiller and panicle morphology, of which tiller angle and leaf angle are two important agronomic traits.

Tiller angle, defined as the angle between the main culm and its side tillers, has long attracted the attention of breeders due to the significant contribution of this trait to plant architecture (Wang and Li, 2008). In cultivated rice, neither a spread-out nor a compact type of plant architecture is beneficial to grain production (Xu et al., 1998). Plants with the spread-out architecture can escape some diseases that are increased by high humidity, but they occupy too much space and increase shading and lodging, consequently decreasing photosynthetic efficiency and grain yield per unit area. On the other hand, compact plants are less efficient in capturing light and are more susceptible to insects and pathogens transmitted by contact. Thus, an appropriate tiller angle is very important to rice production. In determining tiller angle, auxin transport plays an important role. Loss of function of LAZY1 enhanced polar auxin transport (PAT) and impaired lateral auxin transport, resulting in reduced shoot gravitropism and a tiller-spreading phenotype (Li et al., 2007). Suppressing the expression of rice PIN-FORMED1 or enhancing that of rice PIN-FORMED2, two auxin efflux transporters, both altered PAT and increased tiller angles (Xu et al., 2005; Chen et al., 2012). Additionally, many quantitative trait loci (QTLs) affecting tiller angle have been identified (Li et al., 1999). A mutation in the major QTL Tiller Angle Control1 (TAC1) decreased its expression level, leading to the compact tiller in japonica rice, in contrast to the generally wider tiller angle in indica rice (Yu et al., 2007). Tiller angle is also associated with rice domestication. Selection for the PROstrate GROWTH1 (PROG1) mutant caused the critical transition from the prostrate tillers of wild rice (Oryza rufipogon) to the erect tillers of cultivated rice (Jin et al., 2008; Tan et al., 2008). However, the molecular
Leaf angle is the inclination between leaf blade and culm. An erect leaf trait can contribute to rice grain yield by both enhancing photosynthetic efficiency and allowing for increased planting density (Sinclair and Sheehy, 1999; Morinaka et al., 2006; Sakamoto et al., 2006). Leaf angle is formed when the leaf blade bends away from the leaf sheath, during which time cell development on the adaxial side of the lamina joint plays a pivotal role (Duan et al., 2006; Zhang et al., 2009). At present, many related genes have been identified, most of which are involved in the biosynthesis or signaling of the phytohormone brassinosteroids (BRs; Yamamura et al., 2000; Hong et al., 2002, 2003, 2005; Tanabe et al., 2005; Bai et al., 2007; Tong et al., 2009). Other phytohormones, including auxin, ethylene, and gibberellic, also affect leaf angle through their synergistic effects with BRs (Cao and Chen, 1995; Shimada et al., 2006; Song et al., 2009). However, new evidence indicates that novel mechanisms also participate in the determination of leaf angle in rice. For example, Leaf Inclination2 controls leaf angle by inhibiting adaxial cell division but not cell elongation of the lamina joint, distinct from the BR-dependent pathway (Zhao et al., 2010). Recently, another BR-unrelated gene, Increased Leaf Angle1, was reported to affect leaf angle by regulating mechanical tissue formation in the lamina joint (Ning et al., 2011). These findings demonstrate the complicated nature of the rice leaf angle regulatory mechanism.

Most studies of rice plant architecture have only focused on either tiller angle or leaf angle. In practice, the two traits are closely related to one another, and many genes can simultaneously affect both, such as Leaf and Tiller Angle Increased Controller, Brassinosteroid Up-Regulated1, and even LAZY1 and TAC1 (Li et al., 1999, 2007; Wang et al., 2008; Tanaka et al., 2009). In this paper, we described the rice mutant loose plant architecture1 (lpa1), which displays a relatively larger tiller angle and leaf angle due to reduced shoot gravitropism. LPA1 encodes a novel INDETERMINATE DOMAIN (IDD) protein with transcriptional repression and shows a specific expression pattern. Our analyses suggest that LPA1 may be involved in a complicated regulatory mechanism. This study can aid in our general understanding of the gravitropism mechanism and the important role it plays in rice plant architecture formation.

RESULTS

The lpa1 Mutant Displays Loose Plant Architecture

The lpa1 mutant was a naturally occurring mutant isolated from an indica variety, Zhongxian3037. During both the vegetative and reproductive stages, lpa1 always exhibited loose plant architecture with larger tiller angle and leaf angle than those of the wild type (Fig. 1, A and B). We measured the tiller angle at heading date and found that the maximum angle was 17.3° in lpa1 but only 9.8° in the wild type (Fig. 1C). Careful observation showed that the large tiller angle of lpa1 was caused by the more symmetrical growth of the tiller node compared with the wild type (Fig. 1D). We also measured leaf angles: each angle was larger in lpa1 than in the wild type, and this difference was more obvious in older leaves, where the maximum angle of the fourth leaf could reach up to 61.2° in lpa1 but only 27.4° in the wild type (Fig. 1E). This difference was further confirmed by the dynamic change observed in the newly developing leaf (Fig. 1F). Detailed examination revealed that the large leaf angle of lpa1 was caused by a more rapid elongation on the adaxial side of the lamina joint (Fig. 1, G and H). We further analyzed the differences in the lamina joint between the wild type and lpa1 at the cellular level. Longitudinal sections of the adaxial epidermis in the lamina joint showed that the larger lamina joint of the mutant was mainly due to the enhanced cell elongation (Fig. 1, I and J).

In addition, we also observed distinct changes in other characteristics. Compared with the wild type, each internode of the lpa1 mutant became shorter but thicker (Supplemental Fig. S1A). Similarly, grains and
leaves became shorter and wider (Supplemental Fig. S1, B and C). Microscopic observation showed that the wall of culm was thickened (Supplemental Fig. S1, D and E) but that cell size did not change (Supplemental Fig. S1, F–I). The structure of vascular bundles also showed complicated changes (Supplemental Fig. S1, J and K).

The lpa1 Mutant Displays Reduced Shoot Gravitropism

In rice, the lazy1 mutant exhibits a tiller-spreading phenotype resulting from reduced shoot gravitropism (Li et al., 2007). To examine whether lpa1 was also involved in the same process, we analyzed the gravity response of young seedlings. The result revealed that both light- and dark-grown mutant seedlings had a reduced gravity response and could not grow upright eventually (Fig. 2, A–C). However, lpa1 roots showed a normal gravity response (Fig. 2D). These results indicated that LPA1 is only involved in shoot gravitropism in rice.

We also tested the gravity response of coleoptiles using 1-cm-long young shoots grown in the dark. The result showed that lpa1 could grow upright completely, but the time was delayed about 4 h when compared with the wild type (Fig. 2, E–G). This indicated that the mutant coleoptile gravitropism is aberrant in gravity perception or signal transduction but normal in organ bending, according to the gravity response process.

In higher plants, the starch-statolith hypothesis, postulating that the amyloplast sedimentation along the direction of gravity in statocytes is responsible for gravity perception, has been widely accepted (Sack, 1997). We observed amyloplast sedimentation in the basal region of coleoptile where shoot bending occurs. Under gravistimulation by turning young shoots upside down, most wild-type amyloplasts had moved to the basal ends of the coleoptile parenchyma cells along the direction of gravity within 10 min (Fig. 2, H and J). In contrast, the amyloplasts of lpa1 were only located in the middle parts of cells within the same time period, but they reached to the bottom after 20 min (Fig. 2, I and J). This result suggested that LPA1 may affect gravity perception by regulating the sedimentation rate of gravity-sensing amyloplasts in rice coleoptiles.

Map-Based Cloning and Functional Verification

To map LPA1, we generated an F2 population derived from the cross between lpa1 and 02428 (a wild-type japonica variety with compact plant architecture). LPA1 was roughly mapped on the short arm of chromosome 3, linked to sequence-tagged-site (STS) markers S1 and S2. Because the effects of lpa1 are not significantly distinct from natural variation between indica and japonica types, we developed a series of recombinant inbred lines containing the heterozygous LPA1/lpa1 allele from the F2 population, and four lines showing obvious segregation of phenotype were identified in the F5 generation. Using 80 recessive individuals from these lines, we restricted LPA1 to a 570-kb interval between STS markers S6 and S7 and further narrowed the interval to 40 kb between cleaved-amplified polymorphic sequence marker C1 and STS marker S7, using 963 recessive individuals from the F6 generation (Fig. 3A).

There are three putative genes within the 40-kb region. Genomic sequencing revealed an insertion in the third exon of LOC_Os03g13400. We isolated this insertion and confirmed its existence at the transcriptional level (Fig. 3, B and C). Nucleotide BLAST showed that the insertion is a Ty1-copia group retrotransposon (Supplemental Fig. S2), which results in a premature transcriptional termination and a deduced truncated protein with a 203-amino acid C-terminal deletion in the mutant (Fig. 3D).

To investigate the effects of LOC_Os03g13400, an RNA interference (RNAi) vector and an overexpression (OE) vector driven by the cauliflower mosaic virus (CaMV) 35S promoter were constructed and transformed into Yandao8 (a wild-type japonica variety with compact plant architecture). Most RNAi and OE transgenic plants showed loose and compact plant architectures with differing degrees, respectively, compared with Yandao8 (Fig. 4A), from which one typical RNAi plant and one typical OE plant were selected for detailed analysis.

Following two generations of self-pollination, the RNAi and OE transgenic plants showed stable phenotypes. Detailed observation showed that both tiller angle and leaf angle increased in the RNAi plant but decreased in the OE plant (Fig. 4, B and C). Real-time PCR analysis showed that the expression level of LOC_Os03g13400 was down-regulated nearly 4-fold in the RNAi plant but up-regulated more than 20-fold in the OE plant (Fig. 4, D and E). Furthermore, the gravity response was also reduced in the RNAi seedlings (Fig. 4F), and multiple independent RNAi lines provided more solid evidence (Supplemental Fig. S3, A–C). These results strongly confirmed that LOC_Os03g13400 is LPA1 and also showed that the transcription level of LPA1 is closely associated with rice plant architecture.

LPA1 Exhibits a Specific Expression Pattern

We performed 5’ and 3’ RACE, and the results revealed that the full-length complementary DNA (cDNA) of LPA1 is 1,806 bp long with a 1,317-bp open reading frame, a 273-bp 5’ untranslated region, and a 216-bp 3’ untranslated region. Sequence comparison between genomic DNA and cDNA revealed that LPA1 is composed of three exons and two introns (Supplemental Fig. S4).

To define the temporal and spatial expression pattern of LPA1, we performed an analysis of LPA1 transcription using RNA samples from different tissues.
Real-time PCR revealed that \textit{LPA1} was highly expressed in the lamina joint and internodes, especially in young tissues. However, older tiller base also showed a high expression level equivalent to the young second internode (Fig. 5A). \textit{LPA1} was also moderately expressed in coleoptile, root, seedling, and panicle but was barely detectable in leaf blade and leaf sheath (Fig. 5A). The higher expression levels of \textit{LPA1} in the lamina joint and tiller node correspond well with the main phenotypes of the mutant.

In grasses, the leaf sheath pulvinus was identified as a specialized organ for gravity response (Kaufman et al., 1987). We analyzed the expression of \textit{LPA1} in this tissue. To our surprise, \textit{LPA1} was exceptionally abundant in leaf sheath pulvinus, about 35-fold higher than in the coleoptile, strongly suggesting an important role for \textit{LPA1} in leaf sheath pulvinus gravitropism (Fig. 5B). Additionally, we also found that the expression of \textit{LPA1} in dark-grown etiolated seedlings was 1.5-fold higher than it was in those grown in the light (Fig. 5C), indicating that light can inhibit the expression of \textit{LPA1}, consistent with previous reports that light affects gravitropism in plants (Correll and Kiss, 2002; Kim et al., 2011).

\textbf{LPA1 Defines a Novel Subfamily of IDD Proteins}

\textit{LPA1} encodes a predicted 438-amino acid protein. Sequence analysis indicated that \textit{LPA1} is a typical Cys-2/His-2 zinc finger protein (Supplemental Fig. S4) belonging to the plant-specific IDD protein family, where it was also known as OsIDD14 (Colasanti et al., 2006). The IDD protein family was first defined by the maize (\textit{Zea mays}) \textit{INDETERMINATE1} (ID1), and all members share a conserved IDD that has a putative nuclear localization sequence at the N terminus, followed by four distinct zinc finger motifs (sequentially named ZF1, ZF2, ZF3, and ZF4; Colasanti et al., 2006). It is interesting that OsIDD12, OsIDD13, and OsIDD14/\textit{LPA1} are more similar to one another and divergent from the other 12 rice members, like AtIDD14, AtIDD15/\textit{SHOOT GRAVITROPISM5} (SGR5), and AtIDD16 among the 16 Arabidopsis (\textit{Arabidopsis thaliana}) members (Colasanti et al., 2006), suggesting the specificity of the six proteins in the IDD protein family. Sequence alignment revealed that the six proteins shared a unique conserved coiled-coil domain at the C terminal.

\textbf{Figure 2. Gravitropism analysis of the wild type and \textit{lpa1}.} A and B, Four-day-old seedlings of the wild type (WT) and \textit{lpa1} grown in light (A) and dark (B) after a 24-h gravistimulation. Bars = 1 cm. C, The ultimate curved angle of wild-type and \textit{lpa1} seedlings grown in light and dark after a 3-d gravistimulation. Values are means ± s.d. D, Kinetic comparison of the curved angle of root between the wild type and \textit{lpa1} under gravistimulation. Values are means ± s.d. E and F, Coleoptiles of the wild type and \textit{lpa1} after 4 h (E) and 8 h (F) of gravistimulation. Bars = 1 cm. G, Kinetic comparison of the curved angle of coleoptiles between the wild type and \textit{lpa1} under gravistimulation. H and I, Kinetic comparison of the sedimentation of amyloplasts along the direction of gravity in parenchyma cells of coleoptiles between the wild type (H) and \textit{lpa1} (I). Bars = 25 \mu m. J, Average position of amyloplasts in gravistimulated wild-type and \textit{lpa1} parenchyma cells at different time points. Values are means ± s.e. The schematic cell at right was used to determine the position of amyloplasts. Each cell was divided into four equal segments along its length, numbered 0 to 3. When coleoptiles were inverted, amyloplasts moved along the basal-apical axis and their positions were scored. The arrow indicates the direction of gravity (\textit{g}).
terminus but have lost two other conserved domains (MSATALLQKAA and TR/LDFLG), one or both of which the other members possess (Fig. 6; Colasanti et al., 2006). This result further indicated that the six proteins form a distinctive group in the IDD protein family.

To identify other homologs of LPA1, a BLASTP search was performed. The result revealed that there are 27 proteins showing high sequence similarity with LPA1 in higher plants. Multiple sequence alignments showed that these proteins were highly conserved in the ID domain and the coiled-coil domain. Furthermore, most of these proteins also shared several small characterized motifs, of which the two conserved motifs, EL1 (ELQLLP) and EL2 (LQLSIG), that are similar to ethylene response factor-associated amphiphilic repression (EAR) and EAR-like motifs, were the most remarkable (Supplemental Fig. S5; Kazan, 2006). This result clearly demonstrated that LPA1 defines a novel subfamily of IDD proteins with distinct domains and motifs. Moreover, phylogenetic analysis indicated that the 28 proteins were distinctly classified into two clades, corresponding to proteins from monocots and dicots, respectively, with the exception of a single protein from the bryophyte Physcomitrella patens (Fig. 7). However, the four proteins on the LPA1 branch are distinct from the other monocot proteins (Fig. 7). Some of this divergence may result from their unique conserved spacers between the ZF1 and ZF2 motifs, similar to that of maize ID1 (Supplemental Fig. S5; Kozaki et al., 2004).

Figure 3. Map-based cloning of LPA1 and gene information. A, Map-based cloning of LPA1. LPA1 was mapped in a 570-kb interval restricted by STS markers S6 and S7 using 80 recessive individuals. The interval was narrowed to a 40-kb interval on bacterial artificial chromosome AC134229 restricted by STS marker S7 and cleaved-amplified polymorphic sequence marker C1 using 963 recessive individuals. The number under the marker indicates the number of recombinant individuals. LPA1 contains three exons (thick black bars) and two introns (thin gray bars). The triangle indicates the insertion of a retrotransposon. B, PCR amplification of the retrotransposon. The arrows indicate specific bands of the wild type (WT) and lpa1. C, PCR verification of the retrotransposon using cDNA as a template. 5’ and 3’ indicate two primer pairs whose products overlap, partially corresponding to the 5’ and 3’ parts, respectively, of the full-length cDNA of LPA1. The latter harbors the insertion site of the retrotransposon and cannot get the predicted product in lpa1. The arrows indicate specific bands. D, The insertion of this retrotransposon leads to a premature transcriptional termination of LPA1. GTGAG indicates the target site duplication of the retrotransposon.

Figure 4. Functional verification of LPA1. A, Phenotype of wild-type Yanda08, RNAi, and OE LPA1 transgenic plants. B, Comparison of tiller angle among Yanda08, RNAi, and OE plants by vertical observation. Bar = 5 cm. C, Comparison of the angle of the third leaf among Yanda08, RNAi, and OE plants. Bar = 1 cm. D and E, Relative expression levels of LPA1 in RNAi (Ri; D) and OE (E) transgenic plants compared with Yanda08 (WT). Values are means ± se of three individual experiments, and the value of Yanda08 is set as 1. F, Four-day-old seedlings of Yanda08 and RNAi transgenic plants grown in light after a 24-h gravistimulation. The arrow indicates the direction of gravity (g). Bar = 1 cm.
To determine the subcellular localization of LPA1, we transiently expressed GFP alone and the LPA1-GFP fusion protein under the control of the CaMV 35S promoter in onion (Allium cepa) epidermal cells. The green fluorescence of GFP alone was distributed in the nucleus, cytoplasm, and plasma membrane, whereas fluorescence from the fusion protein was observed only in the nucleus, indicating that LPA1 is a nucleus-localized protein (Fig. 8A). The expression of this fusion protein in rice protoplasts also revealed a consistent nuclear localization pattern (Fig. 8B), while OsMADS15/DEP acted as a positive control (Supplemental Fig. S6; Wang et al., 2010).

As LPA1 is a putative transcription factor, we analyzed its transcriptional activity using a dual luciferase reporter (DLR) assay system in Arabidopsis protoplasts (Fig. 9A). The result showed that LPA1 and its N terminus (amino acids 1–232) slightly reduced the relative luciferase activity compared with the GAL4 binding domain negative control. However, the C terminus (amino acids 233–438) significantly inhibited this activity by more than 50%, indicating the presence of a strong repression domain (Fig. 9B).

In plants, EAR and EAR-like motifs are well known for their transcriptional repression activities, and they widely modulate various biological processes (Kazan, 2006). Our analysis has indicated the presence of two conserved EAR-like motifs, EL1 and EL2, within the sequence of LPA1. To minimize the C-terminal repression domain, we examined the transcriptional activities of two short polypeptides containing EL1 (amino acids 262–271) and EL2 (amino acids 301–312), respectively. The results showed that EL1 strongly repressed the expression of the reporter gene by more than 90%, while EL2 only showed a slight repression effect, similar to LPA1 and its N terminus (Fig. 9B). In eukaryotes, transcriptional repressors can be divided into active and passive categories, and active repressors contain an intrinsic repression domain (Thiel et al., 2004). Thus, our results indicated that LPA1 is an active transcriptional repressor.
active transcriptional repressor and that its main repression effect is contributed by an EAR-like motif.

DISCUSSION
The Role of LPA1 Is Distinct from That of LAZY1 in Rice Coleoptile Gravitropism

Gravity is an important environmental factor that controls the growth direction of plants. Roots and shoots grow downward and upward, showing positive and negative gravitropism, respectively (Morita and Tasaka, 2004). This response ensures efficient photosynthesis and nutrition acquisition, which is critical for plant survival (Hangarter, 1997).

Generally, the gravity response can be divided into three steps: gravity perception, signal transduction, and organ bending (Haswell, 2003). In studies on the mechanism of gravitropism, two hypotheses have been proposed. The starch-statolith hypothesis postulates that the sedimentation of starch-filled amyloplasts in the direction of gravity within specific cells (statocytes) is the trigger for gravity perception (Sack, 1991), while the Cholodny-Went hypothesis suggests that an uneven distribution of auxin between the two sides of a curving organ induced by gravity results in differential growth and bending (Firn et al., 2000). Genetic evidence from Arabidopsis indicates that both hypotheses are essentially correct (Blancaflor et al., 1998; Fukaki et al., 1998; Utsuno et al., 1998; Weise and Kiss, 1999; Fujihira et al., 2000; Friml et al., 2002; Ottenschläger et al., 2003).

In this study, we showed that LPA1 is specifically involved in rice shoot gravitropism. The gravitropism of seedlings was impaired partially in the mutant. However, the mutant coleoptile was able to grow completely upright in the opposite direction of gravity, although the process was delayed, suggesting that gravity sensing or signal transduction was disrupted in the coleoptile. Further analysis showed that the sedimentation rate of amyloplast in the mutant coleoptile was significantly slower than that of the wild type. These results indicate that LPA1 may influence gravity perception in the rice coleoptile by regulating the sedimentation rate of amyloplasts, supporting the starch-statolith hypothesis. However, LPA1 may also participate in signal transduction, because some cytoplasm components, such as vacuole and actin filaments, have been reported to affect the sedimentation of amyloplasts and are likely to be involved in the gravity signal in Arabidopsis shoot gravitropism (Morita et al., 2002; Yano et al., 2003; Silady et al., 2004; Saito et al., 2005; Nakamura et al., 2011).

Our results further suggested that the function of LPA1 is distinct from that of LAZY1 in rice coleoptile gravitropism. First, the lazy1 coleoptile has partially lost gravitropism and was unable to grow upright (Yoshihara and Iino, 2007), which is different from
Detailed experiments revealed that enhanced PAT and impaired lateral auxin transport caused an equal distribution of auxin and a reduced gravity response in *lazy1* (Li et al., 2007). This result supports the Cholodny-Went hypothesis, which also indicated that *LPA1* may not affect auxin asymmetry. Second, it was demonstrated that the sedimentation rate of amyloplast appeared to be identical between *lazy1* and its wild type (Abe et al., 1994b; Godbole et al., 1999), suggesting that *LAZY1* is unlikely to participate in gravity perception. Third, the expression of *LAZY1* is not affected by the mutation of *LPA1* (Supplemental Fig. S7). Given these reasons, we proposed that *LPA1* and *LAZY1* function at different stages of coleoptile gravitropism, and *LPA1* may participate in a *LAZY1*-independent pathway.

### LPA1 May Be Involved in a Complicated Transcriptional and Protein-Protein Interaction Network

IDD proteins are members of a zinc finger protein family of transcription factors in higher plants, and several members have been well characterized. Maize ID1 is the first described IDD protein, acting as a transcriptional regulator of floral transition (Colasanti et al., 1998). In Arabidopsis, AtIDD3/MAGPIE, AtIDD10/JACKDAW, and AtIDD8/NUTCRACKER are involved in root development with the GRAS transcription factors SHOOT-ROOT and SCARECROW (Levesque et al., 2006; Welch et al., 2007). AtIDD8 also regulates photoperiodic flowering by modulating sugar transport and metabolism (Seo et al., 2011b). Recently, AtIDD1/ENHYDROUS was shown to promote germination by regulating light and hormonal signaling during seed maturation (Feurtado et al., 2011). In rice, three independent studies have demonstrated that OsID1, the ortholog of maize ID1, plays a pivotal role in rice floral transition (Matsubara et al., 2008; Park et al., 2008; Wu et al., 2008).

In this study, we showed that another rice IDD protein, LPA1, plays an important role in rice shoot gravitropism and defines a novel subfamily of IDD proteins with several unique conserved domains and motifs, from which a C-terminal EAR-like motif (EL1) was identified because of its typical amphiphilic feature. We further demonstrated that this motif was

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**Figure 8.** Subcellular localization of LPA1. Localization of 35S:GFP and 35S:LPA1-GFP in onion epidermal cells (A) and rice protoplast cells (B) is shown. GFP indicates the green fluorescence of proteins, DIC indicates the differential interference contrast phase, and Merged indicates the merging of GFP and DIC. Bars = 10 μm.

**Figure 9.** Transcriptional activity of LPA1. A, Scheme of the constructs used in the Arabidopsis protoplast cotransfection experiment. The reporter contains five copies of GAL4 binding elements (GAL4), a minimal TATA box of the CaMV 35S promoter, the firefly luciferase (LUC) gene, and a nopaline synthase (Nos) terminator. Each effector contains a GAL4 DNA binding domain (GAL4 BD) and part of a coding region (D) of LPA1 promoted by CaMV 35S. A translational enhancer sequence from Tobacco mosaic virus (Ω) is located upstream of the site of initiation of translation. B, Relative luciferase activities of Arabidopsis protoplasts cotransfected with reporter and different effectors. Schemes of deletion mutants of LPA1 are shown at left. All luciferase activities are expressed relative to the value of GAL4 BD alone (set as 100). Values are means ± SD of four independent experiments.
responsible for the major transcriptional repression activity of LPA1. In Arabidopsis, AtIDD8 is a transcriptional activator, and its activation domains also locate to the C terminus (Seo et al., 2011b). Our result for LPA1 not only revealed the diversity of transcriptional activities of IDD proteins but also implied that such activities are mainly conferred by their C termini.

Our further analysis suggested that EL1 may have peculiar features. First, most EAR and EAR-like motifs suppress both intramolecular and intermolecular activities of other transcriptional activators, such as Viral Protein16 (VP16) from Herpes simplex virus (Ohta et al., 2001; Hiratsu et al., 2002; Tiwari et al., 2004); however, our data demonstrated that EL1 could not significantly inhibit the activity of VP16 (Supplemental Fig. S8), implying that EL1 may function as a gene-specific transcriptional repressor (Gaston and Jayaraman, 2003). Second, most full-length repressors containing EAR and EAR-like motifs show strong transcriptional repression activities (Hiratsu et al., 2002), but LPA1 only showed a slight repression effect, whereas EL1 by itself had much higher activity. It could be that the flanking sequence contains an activation domain that counteracts the effect of EL1. However, no such domain was found. Another possibility is that LPA1 may perform the repression effect of EL1 by interacting with other proteins. As evidence, some EAR repressors were indeed involved in protein-protein interactions (Chern et al., 2005; Song et al., 2005; Weigel et al., 2005), while the conserved coiled-coil domain also suggests that LPA1 can be a stable part of some protein complexes (Burkhard et al., 2001). However, yeast two-hybrid analyses did not give a meaningful result. Additionally, LPA1 also possesses putative DNA-binding activity, because the highly conserved ID domain has been shown to recognize DNA sequences (Kozaki et al., 2004; Seo et al., 2011b). Therefore, LPA1 may be part of a complicated transcriptional and protein-protein interaction network that regulates rice shoot gravitropism. More studies are required to investigate this regulatory mechanism further.

LPA1 Is the Functional Ortholog of AtIDD15/SGR5 But with Distinct Features

It is intriguing that AtIDD15/SGR5 belongs to the conserved LPA1-defined subfamily, affects the gravity response of inflorescence stems by regulating the sedimentation of amyloplasts, and plays an important role in the formation of branch angle in Arabidopsis (Tanimoto et al., 2008), quite similar to the function of LPA1 in rice. Moreover, a previous report revealed that OsIDD14/LPA1 and its two paralogs, OsIDD12 and OsIDD13, are more closely related to each other but are more divergent from other members of this family in rice (Colasanti et al., 2006). Furthermore, OsIDD12 and OsIDD13 have the same expression patterns as AtIDD14 and AtIDD16, most abundant in panicle or flower, while LPA1 and SGR5 are mainly expressed in gravitropism-related tissues (Morita et al., 2006; Supplemental Fig. S9), indicating that LPA1 is the functional ortholog of SGR5. They may share a conserved biological function in plant shoot gravitropism.

However, LPA1 also has characteristic features distinct from its Arabidopsis counterpart. First, protein sequence alignment revealed that there are several significant differences between LPA1 and SGR5, such as some amino acid substitutions in the conserved ID domain and coiled-coil domain, the insertions/deletions of two minor motifs (EL2 and SYV/MSS), and the most arresting spacer sequence (Supplemental Fig. S5), which implies that LPA1 might have other peculiar functions in rice. Second, LPA1 affects the development of internodes, leaves, grains, and vascular bundles as well as shoot gravitropism in rice (Supplemental Fig. S1), but similar effects in Arabidopsis were not detected in SGR5 (Morita et al., 2006; Tanimoto et al., 2008). Third, the mutation of SGR5 attenuated significantly the shoot circumnutation in Arabidopsis (Tanimoto et al., 2008). However, this movement is only controlled by the LAZY1-dependent pathway in rice (Yoshihara and Iino, 2007), implying that LPA1 may not participate in circumnutation regulation. Fourth, it was recently reported that AtIDD14 can regulate starch metabolism by means of alternative splicing under different temperature conditions (Seo et al., 2011a). Although SGR5 also affects starch metabolism, its function is obviously divergent from that of AtIDD14. Additionally, AtIDD16 has a similar expression pattern to AtIDD14 (Morita et al., 2006), suggesting that both genes may have similar functions. These facts further support the hypothesis that SGR5 may not function redundantly with its two paralogs in regulating Arabidopsis shoot gravitropism (Morita et al., 2006; Tanimoto et al., 2008). However, our expression pattern analysis revealed that OsIDD13 was also highly expressed in leaf sheath pulvinus (Supplemental Fig. S9). As the loss-of-function mutant for OsIDD13 has not been described yet, it is unknown whether OsIDD13 is also involved in the gravitropism of leaf sheath pulvinus. Therefore, whether LPA1 also has a similar role to SGR5 in regulating rice shoot gravitropism is still in doubt. Further investigations are required to understand the functions of LPA1 and its paralogs.

LPA1 Could Potentially Be Used in Rice Ideal Plant Architecture Breeding

In recent years, some genes that affect rice grain yield have been identified, shedding light on some of the molecular mechanisms underlying ideal plant architecture (Ashikari et al., 2005; Song et al., 2007; Xue et al., 2008; Huang et al., 2009; Jiao et al., 2010; Miura et al., 2010). Gravity is an important regulator of plant architecture. In grasses, because of the short-lived coleoptiles, the leaf sheath pulvinus and lamina joints are
the major gravity-responding organs, which control the growth orientations of seedlings, tillers, and leaf blades (Maeda, 1965; Kaufman et al., 1987; Abe et al., 1994a). In our study, LPA1 showed a specific expression pattern, and the high expression level in the leaf sheath pulvinus and lamina joint suggests that LPA1 mainly functions in the gravitropism of these tissues, corresponding to its limited effect on coleoptile gravitropism. This analysis demonstrated that LPA1 affects rice plant architecture by regulating shoot gravitropism in later developmental stages.

There are several genes that control tiller angle in rice. LAZY1 and PROG1 lead to the prostrate growth of tillers, but the major QTL, TAC1, only slightly enlarges the tiller angle in indica rice. Because of their larger effects, LAZY1 and PROG1 are not very suitable for rice breeding, while TAC1 has been extensively utilized in rice plant architecture breeding owing to its smaller effect on tiller angle (Yu et al., 2007). In our study, LPA1 showed an inclined tiller angle of 17.3°, similar to TAC1 (Yu et al., 2007). Although the equivalent effect made too many difficulties in map-based cloning of LPA1, it revealed the potential utilization of LPA1 in rice plant architecture breeding. Moreover, the transgenic results further demonstrated that rice plant architecture was correlated with the endogenous expression level of LPA1. Therefore, LPA1 is a useful gene for plant architecture modification in rice breeding.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Rice (Oryza sativa) plants were grown in a paddie field in Beijing under natural conditions. For light or dark treatment, germinated seeds were placed on bottomless 96-well plates floating on water and moved to growth chambers (28°C) with or without a 14-h/10-h photoperiod. Seedlings were sampled after 7 d.

Histological Analysis

Tissues were fixed in 50% ethanol, 5% acetic acid, and 10% formaldehyde overnight, followed by a series of dehydration and infiltration steps, and then embedded in Technovit 7100 resin (Heraeus Kulzer). Five-micrometer sections were sliced with a Leica microtome, stained with 0.25% toluidine blue, and observed using an Olympus BX51 microscope.

Gravitropism Assay and Amyloplast Sedimentation Analysis

The gravity response was measured according to the method described previously (Li et al., 2007). Briefly, rice seeds were grown in half-strength Murashige and Skoog medium (pH 5.8) after being debubbled and surface sterilized. Two-centimeter-long roots, 1-cm-long shoots, and 4-d-old seedlings were used for testing. The gravity response was determined by measuring the curved angle after reorienting by 90°. For amyloplast staining, a periodic acid-sterilized. Two-centimeter-long roots, 1-cm-long shoots, and 4-d-old seedlings were sampled after 7 d.

Primer Design and Retrotransposon Identification

PCR-based molecular markers for map-based cloning were developed based on sequence differences between indica variety 93-11 and japonica variety Nipponbare according to published data from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Primer sequences are listed in Supplemental Table S1. Long amplification of the retrotransposon was performed using LA Taq (TaKaRa) and the ID-6F/ID-1R primer pair, and the verification of transcriptional level was performed using 5′ (C1-F/C1-R) and 3′ (C2-F/C2-R) primer pairs. The specific primers are listed in Supplemental Table S2.

Real-Time PCR and RACE Analysis

Total RNA was extracted from various samples using TRIzol reagent (Invitrogen). Two to 3 μg of RNA was treated with RNase-free DNase (Invitrogen), and first-strand cDNA was synthesized to using oligo(dT)20 primer (TaKaRa) and Moloney murine leukemia virus reverse transcriptase (Invitrogen). The expression levels of LPA1 and other genes were analyzed using a CFX96 Real Time System (Bio-Rad) and rice Ubiquitin as an internal control. 5′ and 3′ RACE was performed according to the online protocol from TaKaRa (http://www.takara.bio/>. For 5′ RACE, cDNA was synthesized using the 5′ phospho-labeled primer 5′-RT, treated with RNaseH, and cyclized using T4 RNA ligase, followed by nested PCR with primer pairs 5′-1F/5′-1R and 5′-2F/5′-1R. For 3′ RACE, cDNA was reverse transcribed using the Oligo-dT-Adaptor, then nested PCR was performed using primer pairs 3′-1F/Adaptor and 3′-2F/Adaptor. The specific primers are listed in Supplemental Table S2.

Vector Construction and Rice Transformation

To generate the RNAi vector for LPA1, two copies of the 440-bp 5′ RACE product were repeatedly inserted in opposing orientations into pUCCRNAi to form a hairpin, which was then cloned into pCAMBIA1300-35S. To generate the OE vector for LPA1, a 1,432-bp fragment containing the entire open reading frame of LPA1 was amplified using primer pair ZF-C1F/ZF-C2R and cloned into pCAMBIA1300-35S. Both vectors were introduced into Agrobacterium tumefaciens strain EHA105 through electroporation, and the resulting strains were used to transform the japonica rice variety Yanda08. Rice transformation was performed as described previously (Hiei et al., 1994).

Protein Sequence and Phylogenetic Analysis

Rice and Arabidopsis (Arabidopsis thaliana) protein sequences were obtained from The Rice Annotation Project Database (http://rapdb.dna.affrc.go.jp/) and The Arabidopsis Information Resource (http://www.arabidopsis.org/index.jsp), respectively. Four maize (Zea mays) IOD proteins were obtained through a database search from Maize Sequence (http://www.maizesequence.org/index.html) using LPA1 sequence as a query. The other protein sequences were acquired by searching GenBank (http://www.ncbi.nlm.nih.gov/genbank/) using LPA1 sequence as a query. Protein sequences were aligned using Clustal X 1.83, and the conserved residues were displayed by GeneDoc (http://www.nrbsc.org/gbs/gene/doc/). A phylogenetic tree was constructed using MEGA 4.0 based on the neighbor-joining method. The coiled-coil domain was predicted by SMART (http://smart.embl-heidelberg.de/). The spacers and zinc finger motifs were labeled as described previously (Colesanti et al., 2006).

Subcellular Localization

The coding region of LPA1 was amplified using the primer pair GFP-F/GFP-R (the specific primers are listed in Supplemental Table S2) and cloned into pJIT163-AGFP, generating the LPA1-GFP fusion under the control of the 2× CaMV 35S promoter. GFP and LPA1-GFP plasmids were bombarded into onion (Allium cepa) epidermal cells using a PDS-1000/He particle gun (Bio-Rad) or transformed into rice protoplasts as described previously (Li et al., 2009). After 20 h of incubation at 28°C, GFP fluorescence was observed with a confocal laser scanning microscope (Leica TCS SP5).
Transcription Activity Analysis

The transcriptional activity of LPA1 was analyzed using the DLR assay system in Arabidopsis protoplasts (Hao et al., 2010). The firefly luciferase gene driven by the minimal TATA box of the CaMV 35S promoter following five copies of the GAL4 binding element was used as a reporter. The Renilla luciferase gene driven by CaMV 35S was used as an internal control. Different truncated fragments of LPA1 were amplified (the specific primers are listed in Supplemental Table S2) or synthesized and then fused with the yeast GAL4 DNA-binding domain as effectors, driven by CaMV 35S followed by the translational enhancer ϕ from Tobacco mosaic virus. Arabidopsis protoplast preparation and transfection were based on the protocol for Arabidopsis mesophyll protoplasts (Yoo et al., 2007). For each assay, 6 μg of reporter plasmid DNA, 6 μg of effector plasmid DNA, and 1 μg of internal control plasmid DNA were cotransfected. After incubating for 16 h at 23°C, the relative luciferase activity was measured using the DLR assay system and the GloMax 20-20 luminometer (Promega).

Sequence data from this article can be found in the GenBank database under the following accession numbers: LPA1, JQ681528; Ubiquitin, D12629; OsIDD12, AK119895; OsIDD13, AK288964; LAZ1Y, AK676644; TAC1, AP005682; PROG1, AP005632.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Characteristics of internode, grain, and leaf of the wild type and lpa1.

Supplemental Figure S2. The sequence of the retrotransposon.

Supplemental Figure S3. Five independent RNAi transgenic lines of LPA1.

Supplemental Figure S4. Full-length cDNA of LPA1 and the deduced amino acid sequence.

Supplemental Figure S5. Sequence alignment of 28 full-length IDD proteins of a subfamily defined by LPA1.

Supplemental Figure S6. Subcellular localization of OsMADS15/DEP-GFP in rice protoplast cells.

Supplemental Figure S7. Relative expression levels of LPA1, TAC1, and PROG1 in 7-d-old seedlings grown in different conditions.

Supplemental Figure S8. EL1 cannot significantly inhibit the transcriptional activation activity of VP16.

Supplemental Figure S9. Relative expression levels of OsIDD12, OsIDD13, and OsIDD14/LPA1 in different tissues.

Supplemental Table S1. PCR-based molecular markers for map-based cloning.

Supplemental Table S2. Primer sequences used for functional analysis of LPA1.

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